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The above references will be acknowledged in the text below by indicating their number from the above list shown in brackets.

BACKGROUND OF THE INVENTION

Schizophrenia is a syndrome which encompasses a variety of symptoms including paranoia, auditory hallucination, delusions, catatonia, bizarre behavior and emotional withdrawal. Schizophrenia affects about 1% of the total population. Its economical and social burden on society is enormous since onset occurs in youth thus requiring patients to be under medical and psychiatric supervision for most of their lives. Schizophrenia is therefore one of the most costly diseases in the industrialized world.

Since the biochemical basis of schizophrenia has not yet been elucidated, diagnosis today is still based solely upon psychiatric evaluation. Furthermore, no therapy is currently available for schizophrenia although the symptoms may be ameliorated by neuroleptic drugs.

Many reports have implicated the immune system in the etiology and course of several mental disorders. Serum antibodies which cross-react with brain antigens have been found in the blood of schizophrenic patients[1–6], thus indicating that schizophrenia is also an autoimmune disease[7–9]. Furthermore, platelets have been used as a model for neuronal tissue[10,11] and elevated levels of autoantibodies to platelets have been detected in schizophrenic and demented patients, but not in patients suffering from manic-depressive disorder, depression, personality disorders or schizoaffective disorders[12-14]. An assay for the diagnosis of multi-infarct dementia and dementia of the Alzheimer type was described based on detection of a high level of a platelet associated antibody[15].

A cellular response against autologous platelets was also demonstrated in schizophrenia patients who showed a delayed type hypersensitivity (DTH) reaction when injected with platelets collected from their own blood[16].

It is therefore the object of the present invention to provide a test for the diagnosis of schizophrenia in a subject.

well as individual proteins, polypeptides or peptides among the Pool 2 proteins which are active in eliciting the DTH reaction in schizophrenic patients, are also an aspect of the invention.

The Pool 2 protein preparation prepared in accordance with the invention and used in the diagnostic methods of the invention include proteins purified from the Pool 2 proteins, polypeptides or peptides comprising sequences of such proteins, fractions thereof, as well as proteins, polypeptides or peptides obtained by synthesis or by genetic engineering having a sequence identical to that of the proteins of the Pool 2 proteins.

In accordance with the invention, Pool 2 proteins used in the diagnostic assay of the invention are such which are capable of eliciting DTH activity in an injected individual, the DTH activity being tested by the test known in the art. In short, the Pool 2 proteins are intradermally injected into the tested individual at the forearm or thigh and the reaction at the injection site is evaluated after 24, 48 and 72 hours by measuring the reaction diameter around the induration. As mentioned above, there may be cases in which the time profile of the reaction will differ from the typical time profile of a DTH reaction.

The present invention further provides a kit for use in diagnosis of schizophrenia, comprising said Pool 2 proteins, active protein fractions obtained therefrom, or individual active proteins or peptides, derived from said Pool 2 proteins. Preferably, such proteins are provided in either injectable form or in a form suitable for preparing an injectable formulation, e.g. a lyophilysate. Typically, the kit will be provided with instructions for use or a chart or pictures for guidance of the manner of scoring the results.

Example 2 Preparation of Pool 1 and Pool 2 proteins

Methods

Platelet suspension containing about 20 gr total protein was obtained as in Example 1, and the platelets solubilized with 40 ml of a solution containing 0.5% of the detergents NP-40 and Triton-X-100 for 5 mins. at room temperature with gentle shaking. The solution was then centrifuged at 4000 g for 15 mins. at 20°C. The supernatant was collected, and the pellet was subjected to two further extractions with 10 ml 0.1% Triton-X-100. The three supernatants were combined and Bio-Lyte Ampholyte™ 3/10 (40%) of BioRad was added to a final concentration of 1%. The solubilized proteins were subjected to isoelectric focusing. 60 ml of sample was applied to the Rotofor™ system of BioRad, using 0.1 M phosphoric acid as anode solution, and 0.1 M NaOH as cathode solution. The isoelectric focusing was performed for about 4 hours at 10°C using 10 Watt constant power until the current remained constant for 30 mins. Proteins were divided into two separate groups in accordance with their pI: proteins having a pI in the range of 2-6.5 are referred to as Pool 1 proteins while proteins having a pI in the range of 6.5-9.5 are referred to as Pool 2 proteins. Pool 1 and Pool 2 proteins were harvested separately and diluted 1:50 with PBS.

Example 3 Injection of Pool 1 and Pool 2 proteins

Method

The following four preparations were injected intradermally into the forearm of a schizophrenic patient:

i. Pool 2 proteins (marked as P2)

ii. Pool 1 proteins (marked as P1)

iii. Autologous platelets (marked A)

iv. PBS.

0.1 ml of each above preparation were injected and the preparations were injected at four different injection sites spaced about 10 cm from

each other. The skin reaction at each injection site was monitored 24 h, 48 h and 72 h after injection.

Results

The results are seen in Fig. 1 where (i) is the highest injection site on the arm and (iv) is the lowest.

As seen in the figure, a DTH response measured as explained above, was observed in a schizophrenic patient at the site of injection of Pool 2 proteins and no DTH reaction was seen at the site of P1 injection. Furthermore, the DTH reaction at the P2 site of injection was substantially enhanced as compared to the DTH reaction seen at the site of injection of the autologous platelets. Thus, it is, in most cases, preferred to use a P2 protein preparation obtained from a pool of blood samples obtained from several heterlogous individuals in the diagnostic assay of the invention.

CLAIMS:

1. Use of a protein preparation comprising platelet derived proteins or fractions thereof having an isoelectric point (pI) above about 6.5 and preferably within the range of above 6.5 to about 9.5, for the preparation of an injectable reagent for diagnosis of schizophrenia in an individual by determining a Delayed Type Hypersensitivity (DTH) reaction in said individual following injection of said reagent to the individual.

2. A kit for use in diagnosis of schizophrenia in an individual by detection of DTH reaction in said individual, comprising:

(i) a protein or a fraction thereof prepared from human platelets, said proteins or fractions thereof having a pI of above about 6.5;

(ii) a chart and/or pictures for guidance of the manner of scoring said DTH reaction; and

(iii) instructions for use.

3. A kit in accordance with Claim 6, wherein the proteins or fractions thereof have a pI within the range of above 6.5 to about 9.5.

4. A kit in accordance with Claims 6 or 7, wherein the proteins or fractions thereof are prepared from heterologous platelets obtained from a number of individuals other than the individual to be tested.

5. A kit in accordance with Claims 6 or 7, wherein the proteins or fractions thereof are prepared from autologous platelets obtained from the individual to be tested.

6. A method for the preparation of a reagent for use in diagnosis of schizophrenia in an individual by detecting a DTH reaction in said individual following injection of said reagent to the individual, comprising:

(a) obtaining blood samples from a number of individuals, preparing a pool from said samples and collecting platelets therefrom;

(b) preparing a protein fraction from said platelet preparation comprising proteins or fractions thereof having a pI of above about 6.5.

7. A diagnostic method for determining schizophrenia in a subject comprising:

(a) obtaining a preparation comprising, as an active component, platelet derived proteins or fractions thereof having a pI above about 6.5;

(b) injecting said preparation into a subject; and

(c) examining the subject for the occurrence of delayed type hypersensitivity reaction at the site of the injection, a positive result being a reaction above that which is observed in non-schizophrenic subjects, indicating that the subject has a high likelihood of being schizophrenic.

8. A diagnostic method for determining schizophrenia in a subject comprising:

(a) obtaining a blood sample from a number of schizophrenic and/or non schizophrenic individuals other than the tested subject and collecting platelets therefrom;

(b) preparing a protein fraction from said platelet separation comprising proteins or fractions thereof having a pI of above about 6.5;

(c) injecting said protein preparation into a subject; and

(d) examining the subject for the occurrence of a delayed type hypersensitivity reaction at the site of the injection, a positive result being a reaction above that which is observed in non-schizophrenic subjects, indicating that the subject has a high likelihood of being schizophrenic.

9. A diagnostic method for determining schizophrenia in a subject comprising;

(a) obtaining a blood sample from an individual and collecting platelets therefrom;

(b) collecting proteins or fractions thereof from said platelet sample, said proteins or fractions having a pI of above about 6.5.

(c) injecting said collected proteins or fractions thereof to the tested individual; and

(d) examining the subject for the occurrence of delayed type hypersensitivity reaction at the site of the injection, a positive result being a reaction above that which is observed in non-schizophrenic subjects, indicating that the subject has a high likelihood of being schizophrenic.

10. The method of any one of the previous claims, wherein said proteins or fractions thereof have a pI within the range of above 6.5 to about 9.5.